

What is claimed is:

5           1. A process for producing a stabilized cell for use as an internal control in methods for isolating and identifying rare cells, said stabilized control cell having determinants in common with said rare cells, said process comprising,

          a) redundantly labeling said control cell with at least two fluorescent labels having the same spectral properties;

10           b) contacting said labeled cells with a cell fixative said fixative effecting stabilization of both cellular structure and antigenic moieties present on said control cells;

          c) subsequently removing the excess fixative to promote long-term storage of said control cells, said control cells being physically and biologically stable for a period up to at least six months.

15           2. The process as claimed in claim 1, wherein said cell fixative is selected from the group consisting of paraformaldehyde, formaldehyde, glutaraldehyde, and glyoxal.

20           3. The process as claimed in claim 1, wherein said fluorescent labels are membrane labels selected from the group consisting of long chain lipophilic carbocyanines, long chain lipophilic indocarbocyanines, long chain lipophilic indodicarbocyanines, and analogs thereof, lipophilic aminostyryl dyes, and long chain analogs of C18 rhodamine B and C18 fluorescein dyes.

25           4. The process as claimed in claim 1, wherein said control cells are labeled with an antibody immunologically specific for an antigen present on said cells, said antibody being conjugated to a fluorescent molecule.

5. The process as claimed in claim 1, wherein cellular components of said control cell are labeled with dyes selected from the group consisting of DAPI, Hoechst 33342, acridine orange, rhodamine derivatives, neutral red, and lipophilic BODIPY™.

5 6. The process as claimed in claim 5, wherein said cellular component is selected from the group consisting of nucleic acids, nuclei, lysosomes, golgi apparatus, mitochondria, and endoplasmic reticulum.

7. A process for producing a stabilized cell for use as an internal control in methods for  
10 isolating and identifying rare cells, said stabilized control cell having determinants in common with said rare cells, said process comprising,

a) redundantly membrane labeling said control cell with at least two fluorescent labels having the same spectral properties;

b) contacting said labeled cells with a cell fixative said fixative effecting  
15 stabilization of both cellular structures and antigenic moieties present on said control cells;

c) subsequently removing the excess fixative to promote long-term storage of said control cells, said control cells being physically and biologically stable for a period up to at least to six months, wherein said control cell expresses epithelial cell adhesion molecule (EpCam) on its surface and also expresses cytokeratin intracellularly.

20 8. The process as claimed in claim 7, wherein said cell fixative is selected from the group consisting of paraformaldehyde, formaldehyde, glutaraldehyde, and glyoxal.

9. The process as claimed in claim 7, wherein said membrane dye is selected from the  
25 group consisting of long chain lipophilic carbocyanines, long chain lipophilic indocarbocyanines, long chain lipophilic indodicarbocyanines, and analogs thereof, lipophilic aminostyryl dyes, and long chain analogs of C18 rhodamine B and C18 fluorescein dyes.

10. A stabilized cell for use as an internal control in methods for isolating and identifying rare cells, said control cell having determinants in common with said rare cells, wherein said control cell is labeled redundantly with at least two fluorescent labels having the same spectral properties, and cellular components and antigenic moieties of said control cell have been  
5 stabilized for a period up to at least six months by exposure to fixative.

11. The control cell as claimed in claim 10, suspended in a buoyant density medium.

12. The control cell as claimed in claim 10, wherein said cell fixative is selected from the  
10 group consisting of paraformaldehyde, formaldehyde, glutaraldehyde, and glyoxal.

13. The control cell as claimed in claim 10, wherein said fluorescent labels are membrane labels selected from the group consisting of long chain lipophilic carbocyanines, long chain lipophilic indocarbocyanines, long chain lipophilic indodicarbocyanines, and analogs thereof, lipophilic aminostyryl dyes, and long chain analogs of C18 rhodamine B and C18 fluorescein dyes.  
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14. The control cell as claimed in claim 10, wherein said control cells are labeled with an antibody immunologically specific for an antigen present on said cells, said antibody being  
20 conjugated to a fluorescent molecule.

15. The control cell as claimed in claim 10, wherein cellular components of said control cell are labeled with dyes selected from the group consisting of DAPI, Hoechst 33342, acridine orange, rhodamine derivatives, neutral red, and lipophilic BODIPY™.  
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16. A stabilized cell for use as an internal control in methods for isolating and identifying rare cells, said stabilized control cell having determinants in common with said rare cells, and comprising a detectably labeled membrane, said cells further comprising stabilized cellular

components and antigenic moieties, said stabilization being effected by exposure to a fixative, wherein said control cell is a tumor cell expressing EpCam on its surface and cytokeratin intracellularly.

5           17. The control cell as claimed in claim 16, suspended in a buoyant density medium.

18. The control cells as claimed in claim 16, wherein said cell fixative is selected from the group consisting of paraformaldehyde, formaldehyde, glutaraldehyde, and glyoxal.

10           19. The control cell as claimed in claim 16, wherein said membrane label is selected from the group consisting of long chain lipophilic carbocyanines, long chain lipophilic indocarbocyanines, long chain lipophilic indodicarbocyanines, and analogs thereof, lipophilic aminostyryl dyes, and long chain analogs of C18 rhodamine B and C18 fluorescein dyes.

15           20. The control cell of 16, wherein said membrane is redundantly labeled with at least two fluorescent labels having the same spectral properties.

20           21. The control cell as claimed in claim 16, said cell being an SKBR3 breast cancer cell, further comprising a second detectably labeled surface determinant selected from the group consisting of mammoglobulin, human milk fat globulin, and HER-2/neu.

22. The control cell as claimed in claim 16, said cell being an MCF-7 breast cancer cell, further comprising a second detectably labeled surface determinant which is an estrogen receptor.

25           23. The control cell as claimed in claim 16, said cell being an LNCaP prostate cancer cell, further comprising a second detectably labeled surface determinant selected from the group consisting of PSMA, PSA, and androgen receptor.

24. The control cell as claimed in claim 16, said cell being a CEM T-cell leukemia cancer cell, further comprising a second detectably labeled surface determinant which is a CD4 molecule.

5           25. The control cell as claimed in claim 16, wherein said cell is a Raji B-cell leukemia cell, further comprising a second detectably labeled surface determinant which is a CD19 molecule.

10           26. The control cell as claimed in claim 16, wherein said cell is an SU-DHL non-Hodgkin's leukemia cell, further comprising a second detectably labeled surface determinant which is a CD20 molecule.

15           27. The control cell as claimed in claim 16, said cell being a C32 melanoma cancer cell, further comprising a second detectably labeled surface determinant which is a CD146 molecule.

20           27. A stabilized cell for use as an internal control in methods for isolating and identifying rare cells, said stabilized control cell having determinants in common with said rare cells, and comprising a redundantly labeled membrane, said membrane being labeled with at least two fluorescent labels having the same spectral properties, said cells further comprising stabilized cellular components and antigenic moieties, said stabilization being effected by exposure to a fixative, wherein said control cell is selected from the group consisting of tumor cells, bacterially infected cells, virally infected cells, myocardial cells, and endothelial cells in circulation, and fetal cells in maternal circulation.

25           28. The control cells as claimed in claim 27, wherein said cell fixative is selected from the group consisting of paraformaldehyde, formaldehyde, glutaraldehyde, and glyoxal.

29. The control cell as claimed in claim 27, wherein said fluorescent label is a membrane

label selected from the group consisting of long chain lipophilic carbocyanines, long chain lipophilic indocarbocyanines, long chain lipophilic indodicarbocyanines, and analogs thereof, lipophilic aminostyryl dyes, and long chain analogs of C18 rhodamine B and C18 fluorescein dyes.

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30. The control cell as claimed in claim 27, wherein said control cells are labeled with an antibody immunologically specific for an antigen present on said cells, said antibody being conjugated to a fluorescent molecule.

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31. The control cell as claimed in claim 27, wherein cellular components of said control cell are labeled with dyes selected from the group consisting of DAPI, Hoechst 33342, acridine orange, rhodamine derivatives, neutral red, and lipophilic BODIPY™.

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32. The control cell as claimed in claim 31, wherein said cellular component is selected from the group consisting of nucleic acids, nuclei, lysosomes, golgi apparatus, mitochondria, and endoplasmic reticulum.

33. The control cell as claimed in claim 27, suspended in a buoyant density medium.

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34. A method for detecting and enumerating rare cells in a mixed cell population, the presence of said rare cells in said population being indicative of severity of a disease state, comprising:

a) obtaining a blood sample from a test subject, said sample comprising a mixed cell population suspected of containing said rare cells;

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b) preparing an immunomagnetic sample wherein said biological specimen is mixed with magnetic particles coupled to a ligand which reacts specifically with a determinant of the rare cells, to the substantial exclusion of other sample components;

c) contacting said immunomagnetic sample with at least one reagent which labels

a determinant of said rare cells; and

d) analyzing said labeled rare cells to determine the presence and number of any rare cells in said immunomagnetic sample, the greater the number of rare cells present in said sample, the greater the severity of said disease state, wherein the improvement comprises the addition of a stabilized cell for use as an internal control cell in said method, said control cell having determinants in common with said rare cells and wherein said membrane of said control cell is detectably labeled and cellular components and antigenic moieties of said control cell have been stabilized for a period up to at least six months by exposure to fixative.

35. The method as claimed in claim 34, wherein said rare cell is a cancer cell and said disease state is cancer.

36. The method as claimed in claim 34, wherein said membrane is redundantly labeled with at least two fluorescent labels having the same spectral properties.

37. The method as claimed in claim 34, wherein said membrane label is selected from the group consisting of long chain lipophilic carbocyanines, long chain lipophilic indocarbocyanines, long chain lipophilic indodicarbocyanines, and analogs thereof, lipophilic aminostyryl dyes, and long chain analogs of C18 rhodamine B and C18 fluorescein dyes.

38. The method as claimed in claim 34, wherein said ligand is an anti-EpCam, and said reagent labels an intracellular cytokeratin, said EpCam and said cytokeratin being present in both said rare cell and said control cell.

39. The method as claimed in claim 38, wherein the control cell is an SKBR3 breast cancer cell, further comprising a second detectably labeled surface determinant selected from the group consisting of mammoglobulin, human milk fat globulin, and HER-2/neu.

40. The method as claimed in claim 38, wherein the control cell is a MCF-7 breast cancer cell, further comprising a second detectably labeled surface determinant which is an estrogen receptor.

5           41. The method as claimed in claim 38, wherein the control cell is an LNCaP prostate cancer cell, further comprising a second detectably labeled surface determinant selected from the group consisting of PSMA, PSA, and androgen receptor.

10           42. The method as claimed in claim 38, wherein the control cell is a CEM T-cell leukemia cancer cell, further comprising a second detectably labeled surface determinant which is a CD4 molecule.

15           43. The method as claimed in claim 38, wherein the control cell is a Raji B-cell leukemia cell, further comprising a second detectably labeled surface determinant which is a CD19 molecule.

20           44. The method as claimed in claim 38, wherein the control cell is a SU-DHL non-Hodgkin's leukemia cell, further comprising a second detectably labeled surface determinant which is a CD20 molecule.

          45. The method as claimed in claim 38, wherein the control cell is a C32 melanoma cancer cell, further comprising a second detectably labeled surface determinant which is a CD146 molecule.

25           46. An improved kit for screening a patient sample for the presence of circulating tumor cells, comprising:

          a) coated magnetic nanoparticles comprising a magnetic core material, a protein base coating material, and anti-EpCAM coupled, directly or indirectly, to said base coating



material;

b) at least one antibody having binding specificity for a cancer cell

determinant;

c) cell specific dye for excluding sample components other than said tumor

5 cells from analysis wherein the improvement comprises the addition of a container comprising stabilized cells for use as an internal control, said stabilized control cells having determinants in common with said rare cells, wherein said membrane of said control cell is detectably labeled, and cellular components and antigenic moieties of said control cell have been stabilized for a period up to at least six months by exposure to fixative, said stabilized control cells being  
10 suspended in a buoyant density medium.

47. The kit as claimed in claim 46, wherein the control cell is a SKBR3 breast cancer cell, further comprising a second detectably labeled surface determinant selected from the group consisting of mammoglobulin, human milk fat globulin, and HER-2/neu.

15 48. The kit as claimed in claim 46, wherein the control cell is a MCF-7 breast cancer cell, further comprising a second detectably labeled surface determinant which is an estrogen receptor.

20 49. The kit as claimed in claim 46, wherein the control cell is an LNCaP prostate cancer cell, further comprising a second detectably labeled surface determinant selected from the group consisting of PSMA, PSA, and androgen receptor.

25 50. The method as claimed in claim 46, wherein the control cell is a CEM T-cell leukemia cancer cell, further comprising a second detectably labeled surface determinant which is a CD4 molecule.

51. The method as claimed in claim 46, wherein the control cell is a Raji B-cell leukemia

cell, further comprising a second detectably labeled surface determinant which is a CD19 molecule.

5 52. The method as claimed in claim 46, wherein the control cell is a SU-DHL non-Hodgkin's leukemia cell, further comprising a second detectably labeled surface determinant which is a CD20 molecule.

10 53. The method as claimed in claim 46, wherein the control cell is a C32 melanoma cancer cell, further comprising a second detectably labeled surface determinant which is a CD146 molecule.

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